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Microbial Resistance to Disinfectants Mechanisms and Significance

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MICROBIAL RESISTANCE TO DISINFECTANTS: MECHANISMS AND SIGNIFICANCE

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16. ABSTRACT

Drinking water disinfection provides the final barrier to waterborne transmission of a wide variety of potentially waterborne infectious agents including pathogenic bacteria, viruses, and protozoa. These agents differ greatly in their innate resistance to inactivation by disinfectants, ranging from extremely sensitive bacterial agents to highly resistant protozoan cysts. The close similarity between microorganism inactivation rates and the kinetics of chemical reactions has long been recognized. Ideally, under carefully controlled conditions, microorganism inactivation rates simulate first order chemical reaction rates making it possible to predict the effectiveness of disinfection under specific conditions. In practice, changes in relative resistance and deviations from first order kinetics are caused by a number of factors including microbial growth conditions, aggregation, and association with particulate materials. The net effect of all of these factors is reduction in the effectiveness and predictability of disinfection processes. In order to insure effective pathogen control, disinfectant concentrations and contact times greater than experimentally determined values may be required. Of the factors causing enhanced disinfection resistance, protection by association with particulate matter is the most significant. Therefore, removal of particulate matter is an important step in increasing the effectiveness of disinfection processes.

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ABSTRACT

Drinking water disinfection provides the final barrier to waterborne transmission of a wide variety of potentially waterborne infectious agents including pathogenic bacteria, viruses, and protozoa. These agents differ greatly in their innate resistance to inactivation by disinfectants, ranging from extremely sensitive bacterial agents to highly resistant protozoan cysts. The close similarity between microorganism inactivation rates and the kinetics of chemical reactions has long been recognized. Ideally, under carefully controlled conditions, microorganism inactivation rates simulate first order chemical reaction rates making it possible to predict the effectiveness of disinfection under specific conditions. In practice, changes in relative resistance and deviations from first order kinetics are caused by a number of factors including microbial growth conditions, aggregation, and association with particulate materials. The net effect of all of these factors is reduction in the effectiveness and predictability of disinfection processes. In order to insure effective pathogen control, disinfectant concentrations and contact times greater than experimentally determined values may be required. Of the factors causing enhanced disinfection resistance, protection by association with particulate matter is the most significant. Therefore, removal of particulate matter is an important step in increasing the effectiveness of disinfection processes.

The primary purpose of the disinfection process in drinking water treatment is the control of waterborne diseases through inactivation of any pathogenic microorganisms present in the water. The high degree of success of this process used alone or in combination with other treatment processes is confirmed by the infrequent occurrence of waterborne disease outbreaks in modern times.

Interestingly, the disinfectant concentrations and contact times used by different water utilities vary widely. There are no federal requirements regarding this but several states and advisory groups provide minimum requirements or recommendations. For instance, Washington State regulations require a free chlorine residual of 0.2 mg/L after 30 minutes of contact. Other recommended standards (1) call for 0.2 to 0.5 mg/L free residual chlorine with 30 minutes contact for groundwaters or 120 minutes contact for surface waters. For chloramines, 1 to 2 mg/L with 120 and 180 minutes contact time for ground and surface waters respectively, is recommended. Regulations and recommendations such as these have been developed and formalized over a period of years and are based in part on disinfection data and in part on practices that seemed prudent and consistently produced finished water that would meet coliform indicator standards.

GENERAL CHARACTERISTICS OF WATERBORNE PATHOGENS

The usual waterborne pathogens comprise a diverse group of microorganisms and include bacterial, viral and protozoan species. Some general physical and chemical characteristics of the pathogens are shown in Table 1. They range in size over nearly 3 orders of magnitude and vary greatly in the nature of their surface characteristics. Those factors as well as their

mode of reproduction and life cycles can have important effects on their survival in the environment and resistance to disinfectants. The viruses are extremely small and are strict parasites, relying on specific living host cells for their replication. The bacteria are more than 10 fold larger in diameter and are capable of reproducing independent of living hosts. The protozoa are approximately 10-fold larger than the bacteria. They have a complex life cycle, requiring a living host for completion of a portion of the life cycle and exist outside the host in an inactive resistant cyst form. The viruses have no metabolic processes or cell membranes and other structures while the bacteria and protozoa have these features along with the complex enzyme systems that are typical of cellular organisms.

DISINFECTION AS A KINETIC PROCESS

Inactivation of microorganisms by chemical disinfectants can be considered as having the characteristics of a a first-order chemical reaction in which the slope of the inactivation curve is dependent on the disinfectant type, species and concentration and the microorganism being inactivated. Although actual data often show deviation from first order kinetics, it is a useful concept for evaluating the comparative effectiveness of disinfectants or the comparative resistance of microorganisms. A number of different methods for expressing these comparisons have been proposed. Baumann and Ludwig (2) described a simple approach based on the disinfectant concentration and exposure time required to inactivate a certain proportion of a specific microbial population under specific conditions of temperature and pH.

Based on data available at that time in the literature they prepared curves as shown in Fig. 1 and developed the general equation:

$$C^n t = K$$

in which:

C is the disinfectant concentration (mg/L)

t is the contact time required for a given percentage of kill

n is a positive constant indicating the slope of the inactivation curve for a given microorganism at a given C and t

K is a constant for a specific microorganism at a given water temperature and pH.

Their analysis of these data indicated that the majority of the disinfection curves had a slope of -1. In this case the general equation becomes $C \cdot t = K$. Although this concept was developed in 1962, it received little attention until reexamined and used in a comprehensive review of disinfection data by a National Research Council Committee (3).

The range of C·t values shown by different types of potentially waterborne pathogens for chlorine is shown in Table 2 (4). All of these data are for free chlorine residual at pH 6.0 and 5°C. The C·t values clearly show that different types of pathogens vary tremendously in their comparative resistance to chlorine, ranging over about four orders of magnitude. The data also show that within the protozoan group C·t vary somewhat but the differences are relatively minor as compared to the differences between groups. Similar variability occurs in the enteric bacteria and viruses.

ABERRATIONS FROM PREDICTED INACTIVATION RATES

Ideally, based on the use of the C•t value, one should be able to achieve any desired level of pathogen kill by varying the disinfectant concentration and/or exposure time needed for the particular microorganism and environmental conditions (pH, temperature). However, a number of factors may alter the slope of the inactivation curve or cause deviations from the expected first order inactivation rates. The types of curves that may be seen are shown in Fig. 2. Curves A and B show first order inactivation at different rates. These curves would indicate inactivation of two homogeneous populations with differing inherent resistance to the disinfectant. Curve C shows typical deviation from first order kinetics, characterized by an initial relatively linear inactivation rate with a gradual slowing and "tailing off" of the rate. Curves such as this are typical of nonhomogeneous populations with different inherent resistance or protection of a portion of the population by extraneous factors such as shielding through association with particles or clumps of the organism.

Typically, linear inactivation rates are usually maintained through at least two to three orders of magnitude of inactivation. It is mainly for this reason that the 99% inactivation level is usually used in calculating comparative C•t values. As shown in Table 2, different isolates of the same species (G. lamblia) may vary in their inherent resistance to inactivation. The reasons for these variations are largely unknown as are the reasons for the large differences in the relative resistance among pathogenic bacteria, viruses and protozoan cysts.

Considerable research on the mechanisms by which chlorine and other oxidants inactivate microorganisms has been conducted and a variety of mechanisms have been suggested. Chang (5) observed that greater uptake of chlorine and more rapid inactivation of E. histolytica cysts occurred at low pH's at which the chlorine was present in the undissociated hypochlorous acid form. Kulikovski et al. (6) and others have implicated damage to cell membranes and inhibition of biochemical activities associated with cell membranes as mechanisms of inactivation. Inhibition of specific enzymes and enzyme systems also have been suggested as inactivating mechanisms. Other studies have shown that chlorine physically damages nucleic acids and in viruses both nucleic acids (7) and surface proteins (8) have been proposed as the critical activity sites for halogen disinfectants. Because these disinfectants are powerful oxidizing agents, it is likely that they affect many vital functions, making it very difficult to determine a specific site or function on which they exert their lethal effect.

GENETICALLY BASED ALTERED RESISTANCE

The possible development of genetically based increased resistance to disinfectants by natural selection has also been studied. An early study (9) indicated that Escherichia coli survivors of repeated exposures to chlorine showed enhanced resistance to chlorine. Others (10,11) found no increase in resistance in bacterial cultures repeatedly exposed to chlorine and/or iodine. In a more recent investigation Haas and Morrison (12) showed that E. coli survivers of low degrees of inactivation by chlorine did not show altered sensitivity. Survivors of high degrees of inactivation appeared to be more sensitive to chlorine. They speculated that the enhanced sensitivity

of progeny of chlorine exposed cells might be caused by chlorine induced mutations that caused loss of some factor influential in preventing lethal activity of chlorine such as ability to repair nucleic acid damage or cell envelope damage. Bates et al (13) reported that poliovirus type 1 subjected to repeated chlorine exposure cycles developed increased resistance to chlorine. C·t values (99% inactivation) estimated for their data (see Fig. 3) for virus subjected to 1, 5, and 10 chlorine exposure cycles are 0.54, 0.9 and 1.8 respectively, constituting about a threefold increase in C·T value after ten exposure cycles. The increase in resistance did not proceed incrementally on a consistent basis. Viruses from some subsequent exposure cycles were less resistant than in previous cycles. Altered resistance to chlorine similar to that seen in the case of bacterial resistance to antibiotics has not been demonstrated. The halogen disinfectants appear to be general cytoplasmic poisons that affect many vital functions and the development of genetically based resistance to such a variety of injurious actions seems unlikely.

GROWTH CONDITION EFFECTS

There is increasing evidence that the disinfectant resistance of bacteria grown in laboratory cultures may differ greatly from that of the same species occuring in the environment. Favero and Drake (14) showed that Pseudomonas alcaligenes, a common bacterial contaminant in iodine treated swimming pools could grow well in swimming pool water. Water grown cultures were much more resistant to iodine than the same isolates, grown in laboratory media. Carson et al. (15) showed that Pseudomonas aeruginosa grown in a natural water environment was much more resistant to inactivation by a variety of disin-

fectants including chlorine dioxide, quaternary ammonium compounds, acetic acid, and glutaraldehyde than the same P. aeruginosa isolate grown on conventional laboratory media. Subsequent studies have shown similar effects for E. coli (16), Klebsiella pneumoniae (17), Yersinia enterocolitica (17) and Legionella pneumophila (18,19). The studies using P. alcaligenes (14) and P. aeruginosa (15) and one of the studies involving L. pneumophila (19) were conducted with cultures grown using water as the medium. The data from these studies show essentially first order inactivation rates with the slopes varying according to growth conditions as illustrated by Fig. 4 (19). In this case, water grown L. pneumophila required nearly 10 times as long for equivalent inactivation as agar grown L. pneumophila. In other studies, they showed that watergrown L. pneumophila subcultured on agar media immediately lost the enhanced resistance.

In several of the other studies (16,17,18), chemostat grown cultures were used, with growth rates of the cultures controlled by limiting nutrients. These studies also showed differences in sensitivity to inactivation but the inactivation curves were characterized by initial rapid inactivation followed by "tailing off." The surviving fraction at the lower end of the curves varied according to growth conditions with the highest level of survival occurring when nutrient levels and temperatures were lowest. The reasons for such changes in disinfectant resistance are not known. Changes in cell composition, altered membrane permeability, or formation of protective slime layers have been proposed as mechanisms but definitive evidence is lacking. These studies point up the effects of bacterial growth conditions on bacterial resistance to disinfectants and pose questions about the adequacy of disinfection data based on the use of laboratory cultures of certain pathogenic and

indicator bacteria. However, most of the bacterial pathogens responsible for drinking water related disease outbreaks multiply in the intestinal tract and do not multiply in aquatic environments. Whether or not bacterial pathogens grown in the intestinal tract are more resistant than when grown on laboratory media is not known. Campylobacter jejuni grown in mice appear to be as sensitive to chlorine as laboratory grown cells (Blaser, submitted for publication).

PARTICLE ASSOCIATION EFFECTS

For disinfection to be effective, there must be contact between the disinfectant and the microorganism. With few exceptions, (e.g. L. pneumophila) the major source of pathogens of potential health significance in drinking water is the feces of man or other animals. Thus, these pathogens are initially associated with particulate matter. The fate of these associations and pathogen association with other types of particles is influenced by many factors. Microorganism size is an important factor. Viruses, because of their small size, can be protected from disinfectant contact by much smaller particles than bacteria or cysts. In addition, the viruses, because of their large surface to mass ratios are more influenced by the surface charges involved in sorption phenomena.

Although the concept that certain particles may protect microorganisms from inactivation by disinfectants is logical and indeed provides the major basis for regulating drinking water turbidity, little direct evidence of such protection was available until recently. In general, the results of these studies indicate that the effects of microorganism-particle association on disinfection efficiency is determined by the nature of the association. In the case of viruses and bacteria adsorbed on surfaces of particles such

as clays or inorganic flocs, inactivation rates are unaffected or are affected only minimally (20,21,22,23,24).

In contrast, viruses associated with cell debris (simulating the conditions under which they are produced), feces or wastewater effluent solids are substantially protected (21,25,26). The protective effects of wastewater effluent solids on coliforms is shown in Fig. 5 (21). Again, inactivation rates are initially very rapid followed by a leveling off and survival of a small fraction of the population after very long contact times. A more graphic illustration of the manner in which naturally occurring viral pathogens may be substantially protected is shown in Fig. 6 (27). This electron micrograph shows large aggregates of membrane-associated rotavirus in human stool specimens. It is evident that the viruses in such complexes would be protected from inactivation by disinfectants. It is significant that the types of material that have been shown to offer protection are those materials that constitute the major sources of waterborne pathogens and would be associated with pathogens in contaminated waters.

SIGNIFICANCE FOR WATER TREATMENT

The overall effect of the factors that alter microbial resistance to inactivation by disinfectants is one of making drinking water disinfection processes less effective and less predictable than data derived from laboratory experiments would predict. In order to overcome these uncertainties and build in additional safety factors, the tendency is to use higher disinfectant doses and longer contact times. These practices increase chemical byproduct formation and thereby increase the potential health problems related to halogenated organics.

The most significant enhanced resistance mechanism is that caused by the protective effects of particulate matter. It is the most significant factor for two reasons. First, because of the nature and sources of the particles that offer the most protection, pathogens are very likely to be associated with them. Second, particle association provides the highest degree of increased protection for the microorganisms of those mechanisms studied. Fortunately, this mechanism is also the most amenable to control in water treatment. The particle removal processes used in water treatment (flocculation, coagulation, filtration) when conducted properly, are very effective in achieving high levels of particle removal, including the pathogens themselves. This, in turn, increases the reliability and predictability of the disinfection step. However, there are virus data from a few studies that suggest a physical state can exist that allows low levels of virus to survive the sedimentation, filtration, and disinfection processes as practiced (28,29). Whether more vigorous control of conventional water treatment processes could eliminate these residual viruses or whether this degree of virus contamination poses a health hazard is yet to be determined.

REFERENCES

1. Upper Mississippi River Board of State Sanitary Engineers. Recommended Standards for Water Works. Health Education Service, Albany, N. Y., 1982.

2. Baumann, E. R. and Ludwig, D. D. Free available chlorine residuals for small nonpublic water supplies. J. Am. Water Works Assoc. 54: 1379-1388 (1962).

3. National Research Council. The disinfection of drinking water. In: Drinking Water and Health, Vol. 2 National Academy Press, Washington, D.C., 1980.

4. Hoff, J. C., Rice, E. W., and Schaefer, F. W. Disinfection and the control of waterborne giardiasis. Proc. 1984 Specially Conf. on Env. Eng. Am. Soc. Civil Eng. New York, N. Y. pp. 239-244.

5. Chang, S. L. Destruction of microorganisms. J. Am. Water Works Assoc. 36:1192-1207 (1944).

6. Kulikovsky, A., Pankratz, H. S. and Sadoff, H. L. Ultrastructural and chemical changes in spores of Bacillus cereus after action of disinfectants. J. Appl. Bacteriol. 38:39-46 (1975).

7. O'Brien, R. T. Structural and compositional changes associated with chlorine inactivation of polioviruses. Appl. Environ. Microbiol. 38:1034-1039 (1979).

8. Keswick, B. H., Fujioka, R. S., and Loh, P. C. Mechanisms of poliovirus inactivation by bromine chloride. Appl. Environ. Microbiol. 42:824-829 (1981).

9. Farkas-Himsley, H. Killing of chlorine-resistant bacteria by chlorine-bromine solutions. Appl. Microbiol. 12:1-6 (1964).

10. Favero, M. S. Comparative chlorine resistance of some bacteria of public health significance. M. S. Thesis, Washington State University, Pullman, Wash. (1962).

11. Favero, M. S., Drake, C. H., and Randall, G. B. Use of staphylococci as indicators of swimming pool pollution. Public Health Rep. 79: 61-70 (1964).

12. Haas, C. N. and Morrison, E. C. Repeated exposure of Escherichia coli to free chlorine: Production of strains possessing altered sensitivity. Water, Air, and Soil Pollut. 16:232-242 (1981).

13. Bates, R. C., Shaffer, P. T. B., and Sutherland, S. M. Development of poliovirus having increased resistance to chlorine inactivation. Appl. Env. Microbiol. 34: 849-853 (1977)

14. Favero, M. S. and Drake, C. H. Factors influencing the occurrence of high numbers of iodine-resistant bacteria in iodinated swimming pools. Appl. Microbiol. 14:627-635 (1966).

15. Carson, L. A., Favero, M. S., Bond, W. W., and Petersen, N. J. Factors affecting comparative resistance of naturally occurring and sub-cultured Pseudomonas aeruginosa to disinfectants. Appl. Microbiol. 23:863-869 (1972).

16. Berg, J. D., Matin, A., and Roberts, P. V. Effect of antecedent growth conditions on the sensitivity of Escherichia coli to chlorine dioxide. Appl. Environ. Microbiol. 44:814-819 (1982).

17. Harakeh, M. S., Berg, J. D., Hoff, J. C. and Matin, A. Susceptibiltiy of chemostat grown Yersinia enterocolitica and Klebsiella pneumoniae by chlorine dioxide. Appl. Environ. Microbiol. 49:69-72 (1985).

18. Berg, J. D., Hoff, J. C., Roberts, P. V., and Matin, A. Disinfection resistance of Legionella pneumophila and Escherichia coli grown in continuous and batch culture. In: Water Chlorination: Environmental Impact and Health Effects. Vol. 5 (R. L. Jolley, Ed.), Ann Arbor Science, Ann Arbor, Mich. (1985) In press.

19. Kuchta, J. M., States, S. J., McGlaughlin, J. E., Overmeyer, J. H., Wadowsky, R. M., McNamara, A. M., Wolford, R. S., and Yee, R. B. Enhanced chlorine resistance in tap water - adapted Legionella pneumophila as compared with agar medium-passaged strains. Appl. Environ. Microbiol. 50:21-26 (1985).

20. Stagg, C. H., Wallis, C., and Ward, C. J. Inactivation of clay-associated bacteriophage MS-2 by chlorine. Appl. Environ. Microbiol. 33: 385-391 (1977).

21. Hoff, J. C. The relationship of turbidity to disinfection of potable water. In: Evaluation of the Microbiology Standards for Drinking Water. (C. H. Hendricks, Ed) EPA-570/9-78-00C. U.S. Environmental Protection Agency, Washington, D. C. pp. 103-117. (1978).

22. Boyce, D. S., Sproul, O. J., and Buck, C. E. The effect of bentonite clay on ozone disinfection of bacteria and viruses in water. Water Res. 15: 759-767. (1981)

23. Scarpino. P. V. Effect of particulates on disinfection of enteroviruses in water by chlorine dioxide. EPA-600/2-79-054. U.S. Environmental Protection Agency, Cincinnati, OH.

24. Walsh, D. S., Buck, C. E. and Sproul, O. J. Ozone inactivation of floc-associated viruses and bacteria. J. Environ. Eng. Div., ASCE 106: 711-726 (1980).

25. Sproul, O. J., Buck, C. E., Emerson, M. A., Boyce, D., Walsh, D., and Houser, D. Effect of particulates on ozone disinfection of bacteria and viruses in water EPA-600/2-79-089. U.S. Environmental Protection Agency, Cincinnati, OH (1979).

26. Hejkal, T. W., Wellings, F. M., LaRock, P. A., and Lewis, A. L. Survival of poliovirus within organic solids during chlorination. Appl. Environ. Microbiol. 38: 114-118 (1979).

27. Williams, F. P., Jr. Membrane-associated viral complexes observed in stools and cell culture. Appl. Env. Microbiol. 50: 523-526. (1985).

28. Payment, P., Trudel, M., and Plante, R. Elimination of viruses and indicator bacteria at each step of treatment during preparation of drinking water at seven water treatment plants. Appl. Environ. Microbiol. 49:1418-1428 (1985).

29. Rose, J., Gerba, C. P., Singh, S., Toranzos, G. and Keswick, B. Isolation of entero- and rotaviruses from a drinking water treatment facility. J. Am. Water Works Assoc. In press. (Jan. 1986).

TABLE 1. Some Characteristics of Waterborne Pathogens

Type of Pathogen	Shape	Size (um)	Specific Examples	Nature of Surface
Enteric Viruses	Spher.	0.02-0.07	Hepatitis A Virus Norwalk Agent Gastroenteritis Virus(es)	Protein
Enteric Bacteria	Cylind.	0.3-0.5 x 2-4	Salmonella, Shigella, Escherichia, Campylobacter	Lipopoly-Saccharide Lipoprotein
Enteric Protozoa (cyst form)	Ovoid	5-8 x 10-20	Giardia Entamoeba	Mucopoly-Saccharide?

TABLE 2. Inactivation of Various Microorganisms by Free Chlorine at 5°C, pH 6.0

Microorganism	Chlorine Conc. (mg/L)	Time[a] (min.)	C·t
E. coli	0.1	0.4	0.04
Poliovirus 1	1.0	1.7	1.7
E. histolytica cysts	5.0	18	90[b]
G. lamblia[c] cysts	1.0	50	50
	2.0	40	80
	4	20	80
	8	9	72
G. lamblia[c] cysts	2.5	30	75
G. lamblia[d] cysts	2.5	100	250[b]
G. muris cysts	2.5	100	250[b]

From: Hoff, Rice and Schaefer (4)

[a] for 99% inactivation; [b] extrapolated data; [c] cysts from symptomatic carriers; [d] cysts from asymptomatic carrier

FIG. 1. Inactivation of microorganisms by free chlorine at 20-29 C.

From: Baumann and Ludwig (2)

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Time – min
400
200
100
80
60
40
20
10
8
6
4
2
1
0.01
0.02
0.05
0.1
0.2
0.5
1
2
5
10
20
50
100
FAC – ppm
B. anthracis pH 7.2
B. anthracis pH 8.6
E. histolytica pH 8
E. histolytica pH 7
Coxsackie A2. pH 9
Coxsackie A2. pH 7
Cl – 6
Cl – 10
Cl – 35
Polio pH 8.95-9.25
Hepatitis pH 6.4-6.9
Polio pH 6.88-7.4
Esch. coli pH 8.5
S. dysenteriae pH 7
Esch. coli pH 7

FIG. 2. Effects of microorganism type and state on disinfection survival curves, (A) Sensitive homogeneous populations; (B) more resistant homogeneous population; (C) heterogeneous population or partially protected by aggregation etc.

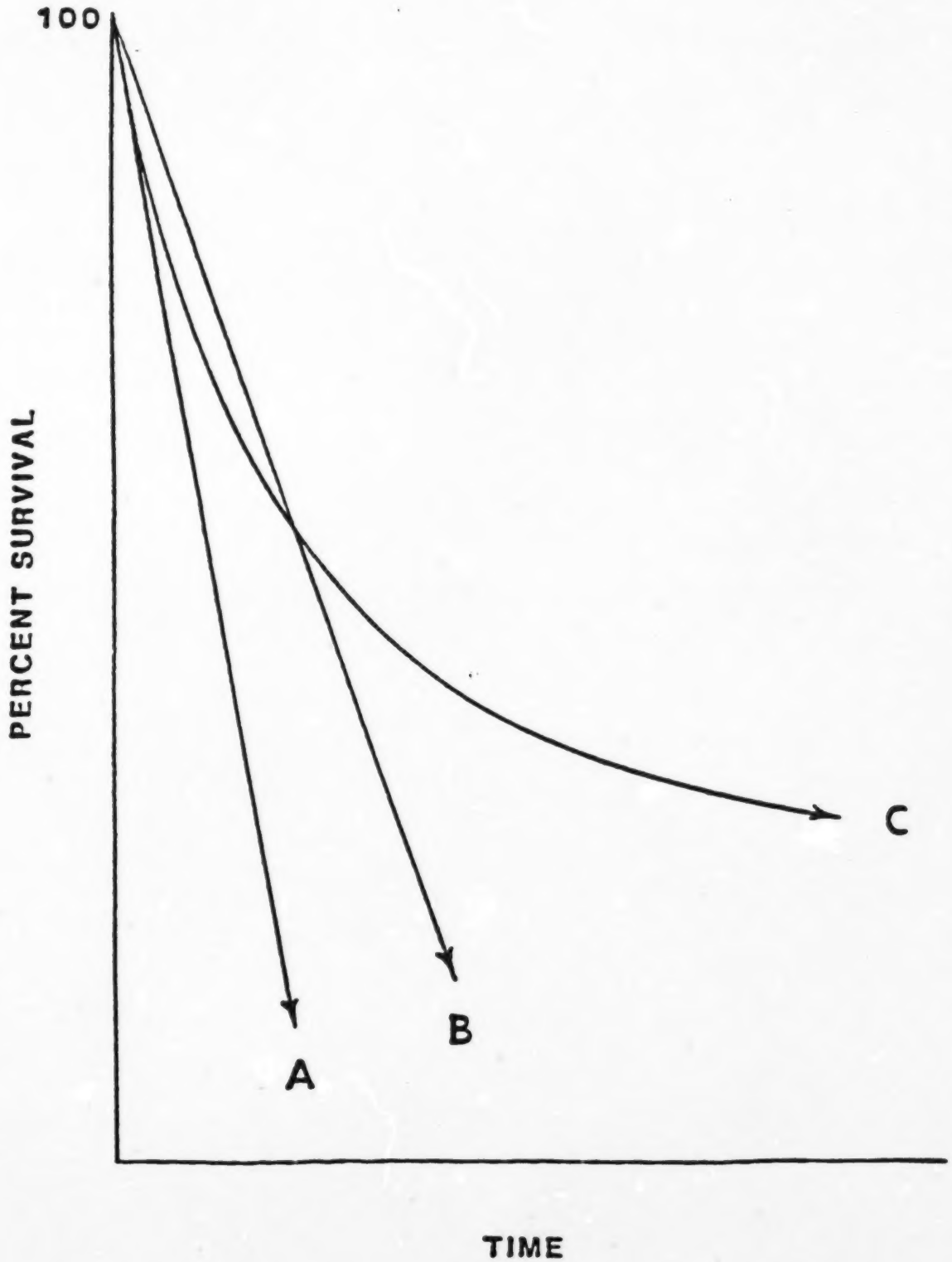
100
PERCENT SURVIVAL
A
B
C
TIME

FIG. 3. Effect of cyclic exposure to chlorine on poliovirus resistance (cycle 1(0); cycle 5 (□); cycle 10 (Δ).

From: Bates *et al.* (13)

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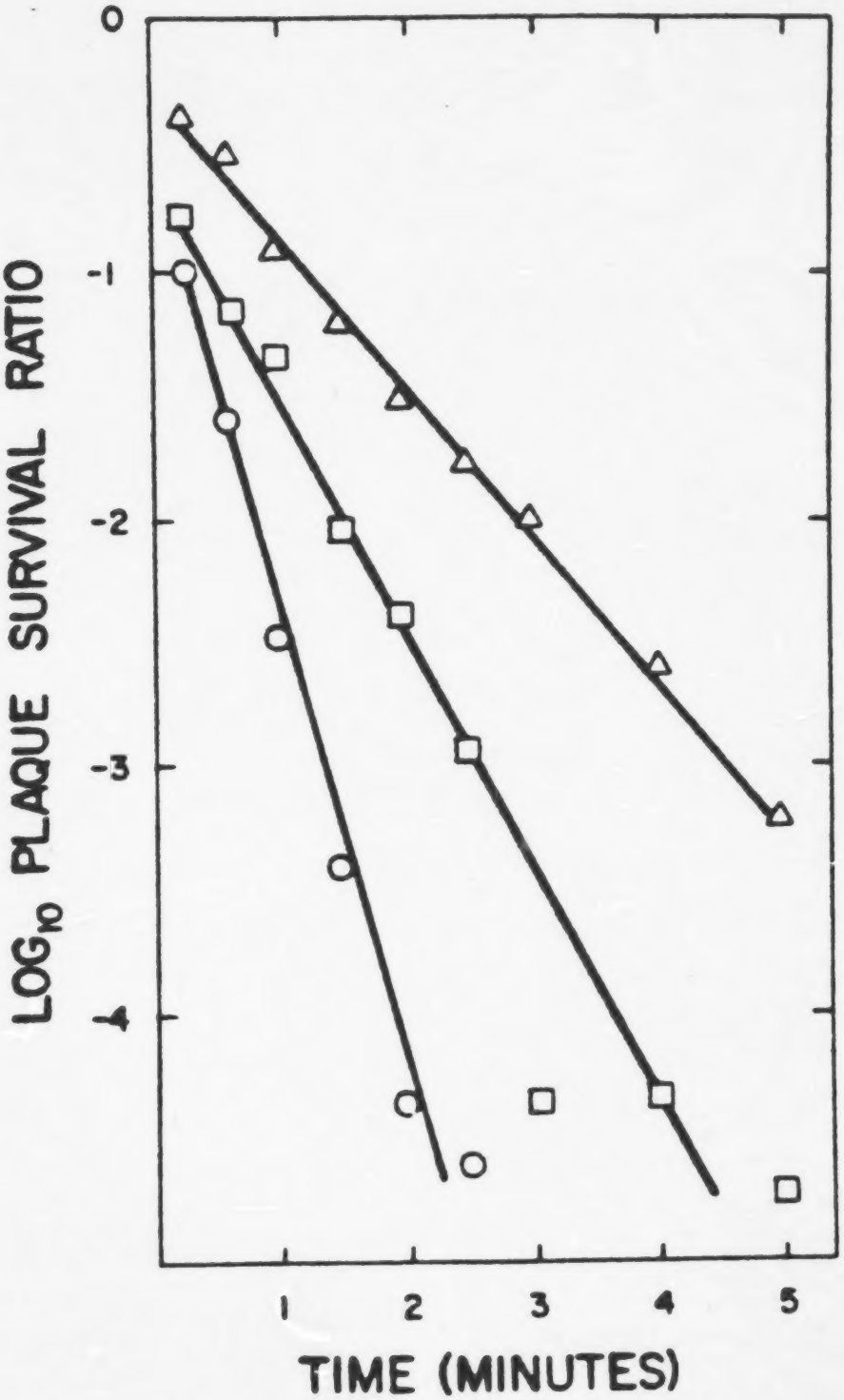
0
-1
-2
-3
-4
LOG10 PLAQUE SURVIVAL RATIO
1
2
3
4
5
TIME (MINUTES)

FIG. 4. Effects of growth conditions on resistance of *L. pneumophila* to chlorine

From: Kuchta *et al.* (19)
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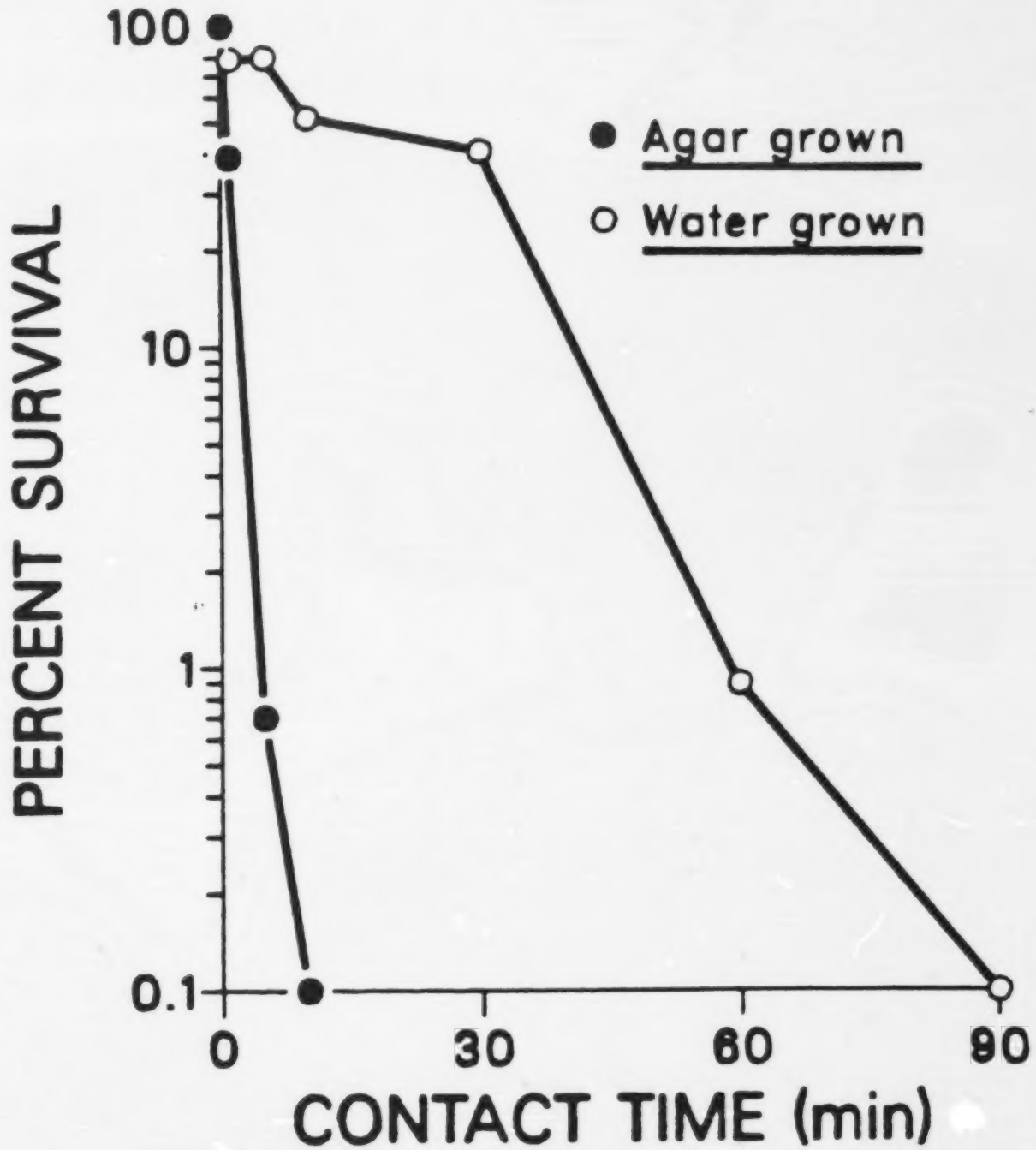
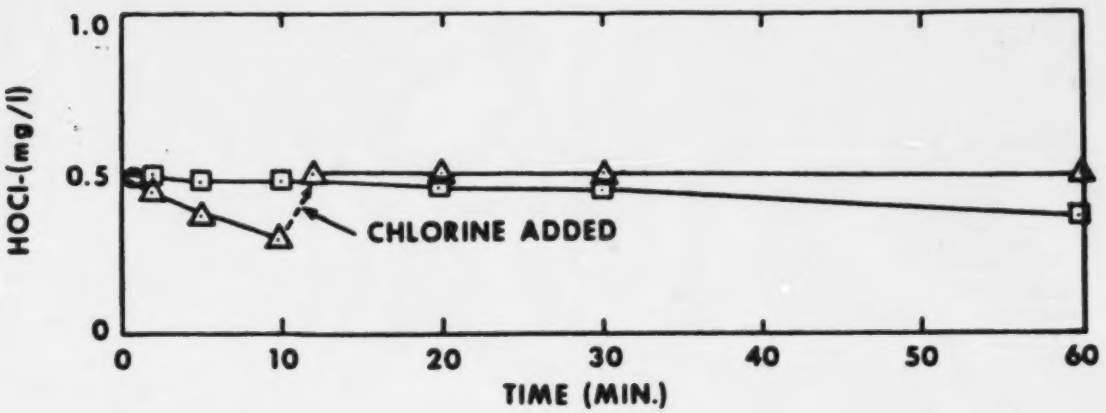

100
10
1
0.1
PERCENT SURVIVAL
Agar grown
Water grown
0
30
60
90
CONTACT TIME (min)

FIG. 5. Inactivation of coliforms associated with primary effluent solids by chlorine (pH 6.0, 5.0° C)

From: Hoff (21)


HOCl-(mg/l)
1.0
0.5
0
CHLORINE ADDED
0
10
20
30
40
50
60
TIME (MIN.)

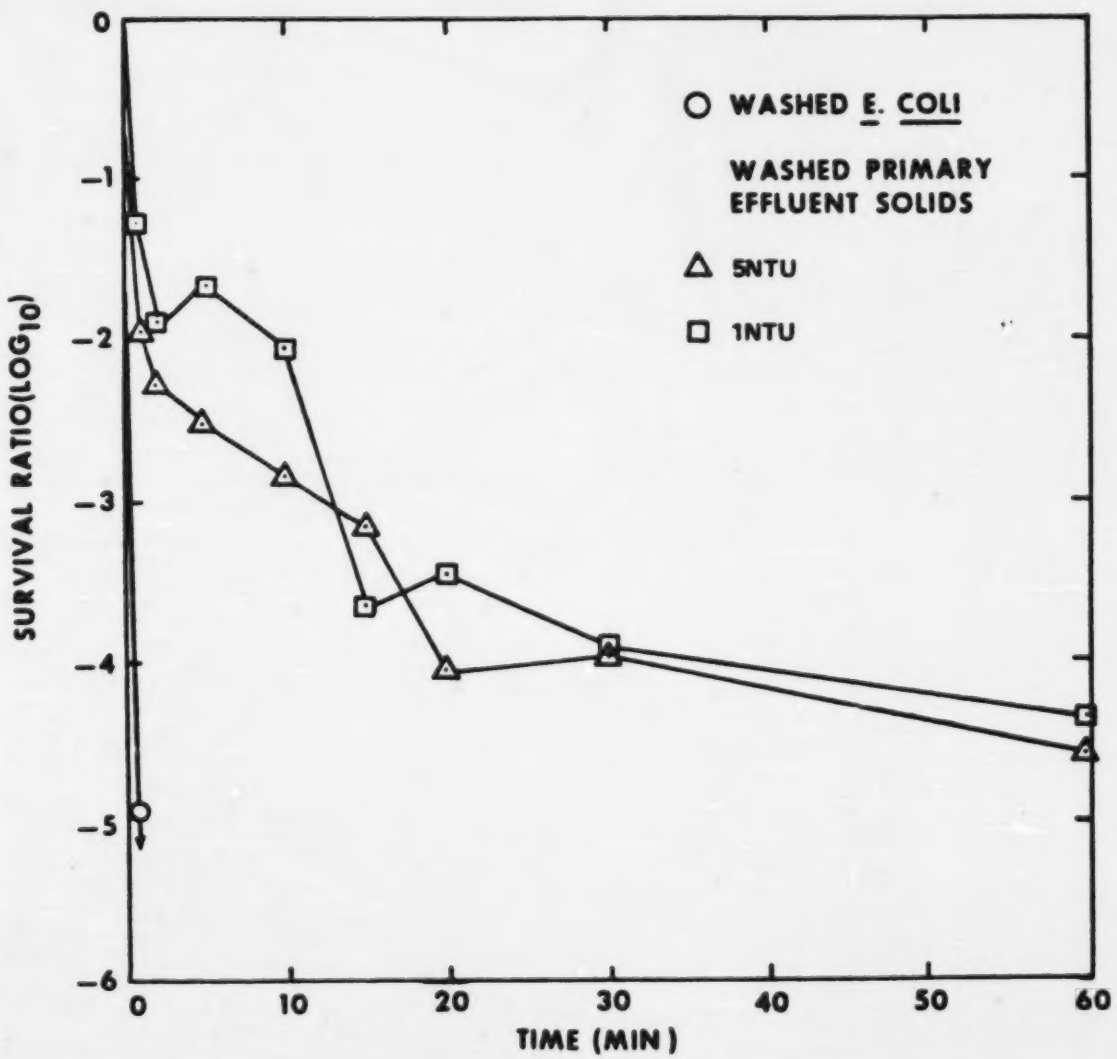

SURVIVAL RATIO(LOG10)
0
−1
−2
−3
−4
−5
−6
WASHED E. COLI
WASHED PRIMARY EFFLUENT SOLIDS
5NTU
1NTU
0
10
20
30
40
50
60
TIME (MIN)

FIG. 6. Membrane-associated virus particles in stool specimens, (a and b) Viral-packet complexes of rotavirus in one specimen, (c through e) Individual enveloped particles (EN), double-shelled particles (SS), and small groups of membrane-associated particles in the same specimen. Bar for panels a through e, 0.2 um. (f and g) Parvovirus-like viral packets observed in another stool specimen, (h) Three Norwalk virus particles associated with a fuzzy membraneous element in another stool specimen. Bar for panels f through h, 0.1 um.

From: Williams, F. L. (27)
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WATER ENGINEERING RESEARCH LABORATORY
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August 19, 1985

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Ms. Linda Illig
Acting Director for Publications
American Society for Microbiology
1913 I Street, N. W.
Washington, D.C. 20006

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   Fig. 2 on p. 853.
2. Kuchta et al: Appl. Environ. Microbiol. 50: 21-26 (1985).
   Fig. 2 on p. 23.
3. Williams, F. P.: Appl. Environ. Microbiol. 50: 523-525 (1985).
   Fig. 1 on p. 524.

Verbal approval recd
from all authors 8/28/85
JCHoff

Thank you,

John C. Hoff, Ph.D.
Research Microbiologist
Microbiological Treatment Branch
Drinking Water Research Division
Water Engineering Research Laboratory

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Date 8-23-85 L. M. Illig

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August 19, 1985

Mr. Joe Zullo
Director of Publications
American Water Works Association
6666 West Quincy Avenue
Denver, Colorado 80235

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1. Baumann and Ludwig: Journal of the American Water Works Association, 54: 1379-1388 (1962).

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Thank you,

Verbal approval received from AWWA 8/28/85. JCHoff

John C. Hoff, Ph.D.
Research Microbiologist
Microbiological Treatment Branch
Drinking Water Research Division
Water Engineering Research Laboratory

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